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Genetic variability in Cantabrian chamois (*Rupicapra pyrenaica parva* Cabrera, 1910)

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Abstract

Genetic variation in 177 Cantabrian chamois (*Rupicapra pyrenaica parva*) from six sampling areas (Cantabrian Mountains, North of Spain) was examined by means of horizontal starch gel electrophoresis. Nineteen enzyme systems were screened in liver and muscle samples, representing a total of 37 presumptive structural loci. Four loci were polymorphic in at least one of the study areas (EST-3, ME, MPI and PT-2). Genetic variability within local samples was low (P = 0.08 and H_e = 0.02). No relevant differences in allele frequencies among these samples were detected, and relatively high gene flow between geographically apparently isolated populations was found (Fst = 0.072 and D between 0.000 and 0.007). These results are discussed with regard to variation of other chamois subspecies.

Introduction

The genus *Rupicapra* Blainville, 1816 occurs in most of the European Mediterranean mountains from the Caucasus to the Cantabrian ranges. Ten subspecies have been described, each one associated to different ranges (Corbet 1978). This isolation might suggest a decrease in genetic variability within populations, with differences expected due to their population history and smaller population size.

The genus was considered monospecific by Couturier (1938). Later, Nascetti et al. (1985) examined the genetic variability of chamois, and clustered populations in the Apennines, Pyrenees and Cantabrian mountains of *R. pyrenaica* Bonaparte, 1845 and the remaining subspecies of *R. rupicapra* Linnaeus, 1758. They found a lower proportion of polymorphic loci and heterozygosity in three Alpine populations (*R. rupicapra rupicapra*) in relation to the Pyrenean sample (*R. pyrenaica pyrenaica*). On the other hand, the Central Apennine population (*R. pyrenaica ornata* Neumann, 1899) showed no genetic variation. However, in samples from Bavarian and Austrian chamois Miller and Hartl (1986) found higher heterozygosity than Nascetti et al. (1985) although some genetic variability parameters did not show this trend.

Cantabrian chamois, *R. pyrenaica parva* Cabrera, 1910, is one of the two subspecies described from the Iberian Peninsula, which is the westernmost part of the distribution range of the genus (Masini and Lovari 1988). Past overhunting caused the original population to fragment into several local populations and some small peripheral ones to become extinct (Nores and Vázquez 1987). In the fifties, population size increased due to the establishment of National Game Reserves (Ortuño and de la Peña 1977). Although

no information exists to quantify previous population changes, the Cantabrian chamois population was divided into two main cores: western and eastern, 15 km apart, and separated by a road, a motorway and a railway. In 1988 population size was 460 (2.1 ind/km^2) and 14 300 (3.1–22.4 ind/km^2) individuals in the western and eastern groups, respectively.

In this study we present data on genetic variability in Cantabrian chamois, the only *pyrenaica* subspecies for which no genetic information is available. We also compare the western and eastern Cantabrian subpopulations, and both subpopulations with the other subspecies previously studied.

Material and methods

Samples of liver and muscle from 177 adult chamois of both sexes (46 males and 131 females) were collected during hunting seasons (August–October) in 1991 and 1992 in the Cantabrian mountains (Asturias, Spain). Sixteen came from Somiedo Game Reserve (western population), and 161 from the following Game Reserves: Aller (N = 24), Caso (N = 50), Picos de Europa (N = 18), Piloña (N = 14) and Ponga (N = 55) in the eastern population (Fig. 1).

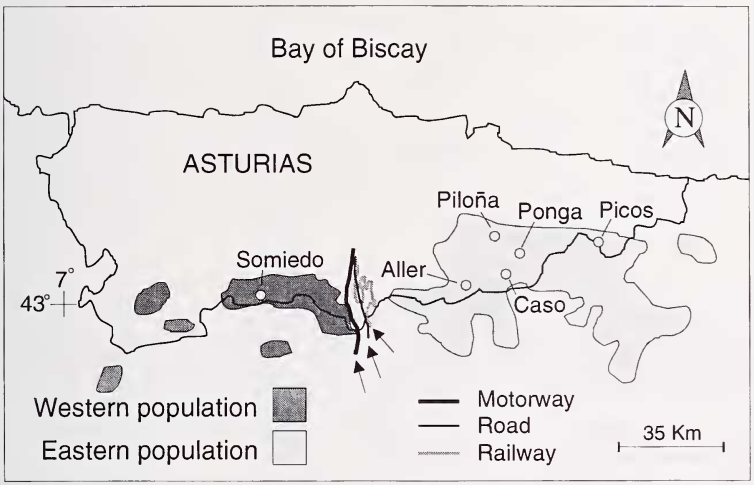

Fig. 1. Present distribution of Cantabrian chamois (shadow areas) in Cantabrian Mountains (North of Spain). Open small circles: sampling areas. Arrows indicate barriers between both populations.

Liver and muscle samples were taken in the field from freshly shot animals, stored at –20 °C during the sampling period and later taken to the laboratory and kept at –74 °C until electrophoretic analyses were carried out. Genetic variability was assessed using horizontal starch gel electrophoresis. Table 1 shows enzyme systems, loci analyzed and some details on study conditions.

Global genetic indices (mean number of alleles per locus, percentage of polymorphic loci and the mean heterozygosity), as well as Wright parameters between populations were estimated using the BIOSYS computer program (Swofford and Selander 1989). We utilized Nei (1978) unbiased genetic distance to compare with other data in the literature where this kind of distance is used, rather than others that might be mathematically more correct (Felsenstein 1984; Hillis and Moritz 1990). We calculated and plotted them using the DISPAN programme (Ota 1993). A Mantel test for the correlation between geographic and genetic distances was performed using the NTSYS program (Rohlf 1993).

Results and discussion

Screening of 19 enzyme systems and unspecified proteins revealed a minimum of 37 presumptive structural loci (Tab. 1). Polymorphism was detected in four of them: EST-3, ME, MPI and PT-2 (Tab. 2). Alleles at a particular locus were identified with regard to relative differences in their electrophoretic mobility compared with the most common one, which is designated "100".

The contingency chi-square analysis of the allelic frequencies did not reveal significant differences except for MPI, basically due to the lower frequency of the "110" allele in the Picos de Europa population.

Table 1. Isoenzyme systems analyzed and some analysis conditions. L = liver, M = muscle, (M) = low enzymatic activity, TME = Tris-maleate-EDTA, TC = Tris-Citrate, LiOH = Lithium hydroxide (Pasteur et al. 1987). To prepare starch gel buffer systems TME and LiOH were diluted twice in relation to original recipes.

Enzyme	E.C. number	Locus	Tissue	Buffer
Aspartate aminotransferase	2.6.1.1	AAT-1	L, M	TME 6.9
		AAT-2	L, M	TME 6.9
Acid phosphatase	3.1.2.2	ACP	L, M	TC 6.7
Adenylate kinase	2.7.4.3	AK	L, M	TC 6.7
Creatine kinase	2.7.3.2	CK-1	L, M	TC 6.7
		CK-2	L, M	TC 6.7
Diaphorase	1.6.2.2	DIA-1	L	LiOH 8.2
		DIA-2	L	LiOH 8.2
Esterases	3.1.1.1	EST-1	L, M	LiOH 8.2
		EST-2	L, M	LiOH 8.2
		EST-3	L, (M)	LiOH 8.2
		EST-4	L, (M)	LiOH 8.2
		EST4MU-1	L	LiOH 8.2
		EST4MU-2	L	LiOH 8.2
α-glycerophosphate dehydrogenase	1.1.1.8	α-GPDH	L, M	TME 6.9
Isocitrate dehydrogenase	1.1.1.42	IDH-1	L, M	TC 6.7
		IDH-2	L, M	TC 6.7
Leucine aminopeptidase	3.4.11.1	LAP	L	LiOH 8.2
Lactate dehydrogenase	1.1.1.27	LDH-1	L, M	TC 6.7
		LDH-2	L, M	TC 6.7
		LDH-3	L, M	TC 6.7
Malate dehydrogenase	1.1.1.37	MDH-1	L, M	TC 6.7
		MDH-2	L, M	TC 6.7
		MDH-3	L, M	TC 6.7
Malic enzyme	1.1.1.40	ME	L, M	TC 6.7
Mannosephosphate isomerase	5.3.1.8	MPI	L, (M)	TC 6.7
Peptidases	3.4.11	PEP-1	L, M	LiOH 8.2
		PEP-2	L, M	LiOH 8.2
Phosphogluconate dehydrogenase	1.1.1.44	6-PGD-1	L, M	TC 6.7
		6-PGD-2	L, M	TC 6.7
Glucosephosphate isomerase	5.3.1.9	PGI	L, M	TME 6.9
Phosphoglucomutase	2.7.5.1	PGM	L, M	TC 6.7
Sorbitol dehydrogenase	1.1.1.14	SDH	L	TME 6.9
Superoxide dismutase	1.15.1.1	SOD-1	L	TME 6.9
		SOD-2	L	TME 6.9
Total proteins		PT-1	L, M	TC 6.7
		PT-2	M	TC 6.7

Table 2. Allelic frequencies at the polymorphic loci found in six Cantabrian chamois populations investigated. Relative migration distances were used for designating alleles.

	Aller	Caso	Picos de Europa	Piloña	Ponga	Somiedo
Est-3 100	1.000	1.000	1.000	1.000	0.958	1.000
Est-378					0.042	
Me 100	0.977	0.980	0.944	1.000	0.981	0.969
Me 89	0.023	0.020	0.056		0.019	0.031
Mpi 100	0.370	0.551	0.833	0.679	0.531	0.500
Mpi 110	0.630	0.449	0.167	0.321	0.469	0.500
Pt-2 100	0.875	0.597	0.667	0.900	0.724	0.708
Pt-291	0.125	0.403	0.333	0.100	0.276	0.292

The observed heterozygosity (Ho) from the Cantabrian western sample was 0.026, higher than the average of the samples from the Cantabrian eastern group (mean = 0.019, range = 0.009–0.033) (Tab. 3). Two out of the 18 tests for agreement between observed frequencies and expected allelic were significant. In both cases, the disequilibrium was due to a deficiency of heterozygotes.

The percentage of polymorphism was P = 8.1 for both groups, varying between 5.4 and 10.8 in the eastern group (Tab. 3). The number of alleles per locus was 1.1 in all cases.

Wright's parameters for the six populations: $F_{IS} = 0.117$, $F_{IT} = 0.181$ and $F_{ST} = 0.072$, indicated a slight deficiency of heterozygotes (F_{IS} positive) and relatively high gene flow

Table 3. Genetic variability values in six Cantabrian chamois populations. N = number of individuals; A = mean number of alleles per locus; P = polymorphism (criterium 99%); H_o = observed heterozygosity; H_e = expected heterozygosity. Groups: Western, western Cantabrian population; Eastern, eastern Cantabrian population (see "Introduction")

Groups	Locality	N	A	P	H_o	H_e
Western	Somieda	14.9(0.4)	1.1	8.1	0.026 (0.017)	0.027 (0.018)
Eastern	Aller	22.0(0.6)	1.1	8.1	0.019 (0.012)	0.020 (0.014)
Eastern	Caso	47.2(0.6)	1.1	8.1	0.033 (0.023)	0.028 (0.019)
Eastern	Picos de Europa	14.7(0.8)	1.1	8.1	0.009 (0.007)	0.025 (0.016)
Eastern	Piloña	12.8(0.4)	1.1	5.4	0.011 (0.008)	0.018 (0.013)
Eastern	Ponga	48.9(1.0)	1.1	10.8	0.025 (0.017)	0.028 (0.017)

Table 4. Nei (1978) genetic distances among the six Cantabrian chamois populations analyzed

	1	2	3	4	5	6
1 Aller	0.0000					
2 Caso	0.0025	0.0000				
3 Picos de Europa	0.0054	0.0008	0.0000			
4 Piloña	0.0018	0.0023	0.0004	0.0000		
5 Ponga	0.0009	0.0002	0.0012	0.0008	0.0000	
6 Somiedo	0.0004	0.0000	0.0013	0.0009	0.0000	0.0000

for all the samples, since only 7.2% of the genetic variability would be due to interpopulation differences, considering the possibility of an interruption in gene flow between the two subpopulations caused by the physical boundary cited. This agreed with the Nei (1978) distances obtained, which were very small between Somiedo (western group) and the Ponga or Caso samples (eastern group), reaching a maximum of 0.005 between the Picos de Europa and Aller populations, both belonging to the eastern group (Tab. 4).

Cantabrian chamois shows a lower genetic variability than that observed by Miller and Hartel (1986, 1987) in the western Alps, or by Nascetti et al. (1985) in the Pyrenees, but higher than that found by these authors in the eastern Alps and the Apennines (Tab. 5).

Table 5. Mean of genetic variability values in different chamois populations. Loci = number of loci studied. Symbols as in table 3

Taxa	Locality	H_e	P	A	Loci	Authors
Rupicapra rupicapra rupicapra	Lower Austria	0.065	0.097	1.097	41	Miller und Hartl (1986)
R. r. rupicapra	Bavaria	0.046	0.170	1.170	41	Miller und Hartl (1986)
R. r. rupicapra	Alps	0.041	0.140	1.192	41	Miller und Hartl (1987)
R. r. rupicapra	Alps	0.012	0.12	1.12	25	Nascetti et al. (1985)
R. pyrenaica pyrenaica	Pyrenees	0.033	0.20	1.20	25	Nascetti et al. (1985)
R. p. ornata	Apennines	0.0	0.0	1.0	25	Nascetti et al. (1985)
R. p. parva	Cantabrian Mts.	0.024	0.08	1.1	37	Present study

Excluding the results on *R. pyrenaica ornata*, where no polymorphism was detected (Nascetti et al. 1985), polymorphism in Cantabrian chamois is the lowest in the genus. However, our mean heterozygosity value exceeds those of one sample of Alpine chamois (Nascetti et al. 1985). These differences between both genetic variability parameters may be due to their different response to a reduction of population size. Leberg (1992) pointed out that the proportion of polymorphic loci and the mean number of alleles per locus are more sensitive to changes in bottleneck events than heterozygosity. On the other hand, when population size increases from a small size, the mean number of alleles per locus recovers sooner than heterozygosity (Nei et al. 1975). Decreasing size and population restoring events may, thus, explain the differences between both parameters.

Genetic distances between western and eastern groups were not higher than those within the eastern group. Therefore, strong genetic uniformity appears to exist in the Cantabrian populations. Moreover, the proposed tree representing these distances did not show any geographical structure. A Mantel test between geographic and genetic distances showed no correlation ($r = -0.17$, $p = 0.29$). This is consistent with F_{ST} values, which show that gene flow is relatively high although a current exchange of individuals between both groups does not seem very probable along this century (period in which a railway, a road and a motorway were built between both groups). Following Wright (1969), and supposing that drift and migration are balanced, $F_{ST} = 1/(4\,Nm + 1)$ (where Nm is the number of individuals dispersed per generation). In our case the value $F_{ST} = 0.072$, would correspond to 3 emigrants per generation. Similar values were obtained by Ryman et al. (1980), and Mccullough and Chesser (1987) in *Alces alces* and *Cynomys mexicanus*, respectively. Gene flow among groups would involve no discrimination between them and no inbreeding within them (Greenwood 1980; Schwartz and Armitage 1980; Melnick 1987; Chesser 1991). Gaillard (1992) indicated that for supporting gene flow in chamois, an interchange of approximately one individual in every two years would be necessary (taking 6.24 years as generation time). In this regard, there may be some genetic connection between western and eastern groups, and the assumed barrier between both has probably not precluded present or very recent migration.

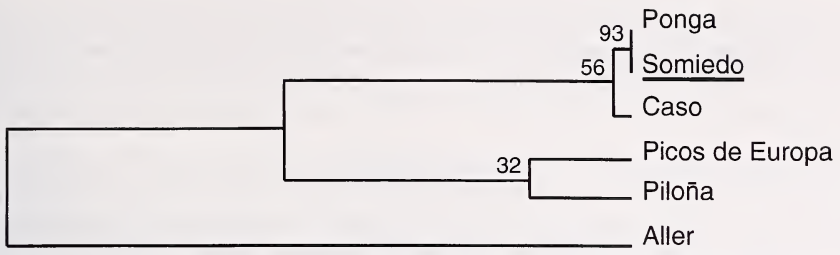

Fig. 2. UPGMA based on Nei (1978) genetic distances. The numbers refer to the percentage each dichotomy appears in 1 000 Bootstrap repetitions. The locality from the western population is presented underlined.

Among the Cantabrian study populations no fixed marker alleles were found; only allelic frequency varied slightly, causing genetic distances between 0 and 0.005, lower than those found by Miller and Hartl (1987) in *Rupicapra rupicapra rupicapra*, and more similar to those indicated by Pemberton et al. (1989) for the same subspecies. The Ponga population showed a marker allele (EST-3*78), with a frequency of 4.2%. This allele may not have been detected in the other populations due to random sampling. Furthermore, the Ponga population is not geographically isolated from the other eastern group populations, and the gene flow for this set of samples was also high.

No important loss of alleles was detected among sets of samples or between western and eastern groups. If previous isolation events among subpopulations had taken place when the population size of Cantabrian chamois was small, alleles could have been lost through genetic drift. However, a later population increase and a recovery of the sympatric situation could have led to partial reconstruction of the original gene pool. A similar process has been described in Lower Austria, where at the end of the past century a chamois population suffered from a bottleneck and probably regained some lost alleles through a subsequent contact with chamois from the Mediterranean Alps (Miller and Hartl 1986).

The loss of alleles in Cantabrian chamois as compared to Pyrenean and Alpine populations must have happened before the disconnection of the two Cantabrian groups, which probably only occured in this century, because it is unlikely that the western and eastern Cantabrian groups lost the same alleles independently.

Miller and Hartl (1986) argued that the *rupicapra* group is not less variable than *pyrenaica* as stated by Nascetti et al. (1985), and that the differences may be due to the different enzyme systems analysed in both studies: "Most loci which were found to be polymorphic in our populations were not investigated by Nascetti et al. (1985)" (Miller and Hartl 1986). However, we point out that these non-examined loci may be polymorphic, both in the Pyrenean and Alpine populations surveyed by Nascetti et al. (1985), but this could not explain why the first population has 2-fold polymorphism and heterozygosity values. Thus, it is also possible that sample size, population history or hunting management (Ryman et al. 1981) could have caused the differences between these populations. Nevertheless, valid comparisons should be based on the same enzymatic systems and similar sized samples (relative to population size).

The comparison between our study and the above mentioned ones showed that we studied 15 of the 17, and 17 of the 25 enzymatic systems analyzed by Nascetti et al. (1985) and Miller and Hartl (1986, 1987) respectively. Moreover, we screened all the polymorphic systems found in Nascetti et al. (1985) but only 3 of the 6 in Miller and Hartl (1986) and 4 of the 8 in Miller and Hartl (1987). Thus, the comparison seems to be more pertinent to the Nascetti et al. (1985) study.

On the other hand, in our study, the relative sample size of western and eastern groups was different (Fisher exact test, $p < 0.001$). Western and eastern group sample sizes in relation to the size of each subpopulation were 3.57% and 1.06%, respectively, but we did not find any relation between genetic variability and sample size in the average of the two groups.

Our results do not indicate a bottleneck event in the western group. The proportion of polymorphic loci is not lower than in the eastern group, as would be expected after a bottleneck situation. Although western group population size (420 individuals) is similar to the *R. pyrenaica ornata* population (350–400 individuals, Lovari 1988), their histories seem to be different. The population size of *R. p. ornata* was only 40 individuals 35 years ago (Lovari 1977); however, we do not know the minimum number of animals in the western group after it was considered to be isolated from the rest of the *R. p. parva* population. 176 individuals censured in 1975 is the oldest available information about western population (Ortuño and de la Peña 1977). It is possible that western group size did not decrease enough to cause a substantial loss of alleles, or that genetic variability have been mantained by immigration from the eastern group in accordance with the Wright parameters obtained. However, current western and eastern group sizes are larger than in the forties and displacements have not been verified between both groups. Therefore, displacements probably did not occur when both groups were smaller. A similar genetic structure in the western and eastern group suggests an exchange between them, perhaps by colonizer males. This would impede full isolation, genetic divergence and inbreeding within groups.

Should the supposed physical barriers between the two groups of the current population become effective, subdividing the population, the effect would probably take a long time to be detected by this method.

It would be interesting to use other molecular techniques on more variable genes or DNA regions to verify that the observed gene flow is sufficient to maintain this population as a single evolutionary unit.

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Zusammenfassung

Genetische Variabilität bei der Kantabrischen Gemse (Rupicapra pyrenaica parva Cabrera, 1910).

Die genetische Variabilität von 177 Kantabrischen Gemsen (*Rupicapra pyrenaica parva*) aus sechs Probengebieten (Kantabrisches Gebirge, Nordspanien) wurde mittels horizontaler Stärkegelelektrophorese von Leber- und Nierenextrakten untersucht. Bei 19 Enzymsystemen konnten insgesamt 37 hypothetische Strukturgenloci ausgewertet werden. Vier Loci (EST-3, ME, MPI und PT2) zeigten einen genetischen Polymorphismus. Die genetische Variabilität innerhalb der Probengebiete war gering ($P = 0.08$, $He = 0.02$). Gleiches gilt für die genetische Differenzierung zwischen den Beständen

(Fst = 0.072, D 0.000–0.007), wobei auch zwischen geographisch eher isolierten Populationen ein relativ hoher Genfluß festgestellt wurde. Die Ergebnisse werden unter Bezugnahme auf die genetische Variation bei anderen Unterarten der Gemse diskutiert.

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